

CLAIMS

1. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by a nucleic acid sequence selected from the group consisting of the *Staphylococcus aureus* open reading frames (ORFs) listed in Table 1.
2. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by an essential or important nucleic acid sequence selected from the group consisting of the *Staphylococcus aureus* open reading frames (ORFs) listed in Table 1, wherein said essential or important nucleic acid sequence is identified as being essential or important by integration knock-out coupled with extra-chromosomal complementation.
3. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by an essential or important nucleic acid sequence selected from the group consisting of the *Staphylococcus aureus* open reading frames (ORFs) listed in Table 1, wherein said essential or important nucleic acid sequence is identified as being essential by integration of a regulatable promoter into the gene.
4. A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the bacterial gene of claim 1.
5. A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the bacterial gene of claim 1.
6. A method screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 2.
7. A method screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 2.

8. A method of screening for an antibacterial agent comprising determining whether a test compound is active against the essential or important bacterial gene of claim 3.

9. A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 3.

10. The method of claim 5, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with test compound;

and

b) determining whether said test compound binds to said protein or said fragment;

wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

11. The method of claim 7, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound;

and

b) determining whether said test compound binds to said protein or said fragment;

wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

12. The method of claim 9, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound;

and

b) determining whether said test compound binds to said protein or said fragment;

wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

13. A method for evaluating a test agent for inhibition of expression of the gene of claim 1, comprising:

- a) contacting a cell expressing said gene with said agent; and
 - b) determining the amount or level of expression of said essential gene in said sample.
14. A method for evaluating test agent for inhibition of expression of the essential or important gene of claim 2, comprising:
- a) contacting a cell expressing said essential or important gene with said agent; and
 - b) determining the amount or level of expression of said essential or important gene in said sample.
15. A method for evaluating a test agent for inhibition of expression of the essential or important gene of claim 3, comprising:
- a) contacting a cell expressing said essential or important gene with said agent; and
 - b) determining the amount or level of expression of said essential or important gene in said sample.
16. The method of claim 13, wherein said level of expression is measured by measuring the amount of expression product in said cell relative to a cell that has not been contacted with said agent.
17. The method of claim 13, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.
18. The method of claim 13, wherein said level of expression is measured by measuring the level of expression of a protein fusion to said gene relative to a cell containing said protein fusion that has not been contacted with said agent.
19. A method for evaluating a potential antibacterial agent, comprising the steps of:
- a) providing a bacterial strain comprising a mutant form of the gene of claim 1, wherein said mutant form of the gene confers a growth conditional or attenuated growth phenotype;

b) contacting bacteria of said bacterial strain with said test compound is semi-permissive or permissive growth conditions; and

c) determining whether the growth of said bacterial strain comprising said mutant form of a gene is reduced in the presence of said test compound to a greater extent than a comparison bacteria comprising a normal form of said gene.

20. A library of nucleic acid sequences consisting essentially of nucleic acid sequences having at least about 80% protein sequence identity to a nucleic acid sequence selected from the group consisting of the *Staphylococcus aureus* open reading frames (ORFs) listed in Table 1, wherein said library of nucleic acid sequences is employed to identify essential genes in *Staphylococcus*.

21. A map of at least about 500-1500 transposon insertions in the genome of *Staphylococcus aureus*, wherein said map is useful for identifying genes that are essential for survival of said *Staphylococcus aureus*.

22. A vector comprising a promoter operably linked to the nucleic acid sequence of claim 1.

23. The vector of claim 22, wherein said promoter is active in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Hemophilus influenzae*, *Neisseria gonorrhea*, *Klebsiella pneumoniae*, and *Streptococci*.

24. A host cell comprising the vector of claim 22.

25. A fragment of the nucleic acid of claim 1, said fragment comprising at least 10, at least 20, at least 25, at least 30, or at least 50 consecutive bases of said nucleic acid.

26. A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 1.

27. A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 2.

28. A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 3.

29. An antibody or antibody fragment capable of specifically binding the protein of claim 26.
30. An antibody or antibody fragment capable of specifically binding the protein of claim 27.
31. An antibody or antibody fragment capable of specifically binding the protein of claim 28.
32. An agent identified as having anti-bacterial activity by any of the methods of claims 4-19.
33. A method for inhibiting the growth or survival of *Staphylococcus aureus* comprising contacting said bacteria with the agent of claim 32 so as to inhibit growth or survival.
34. A pharmaceutical composition comprising the agent of claim 32.
35. A method for treating a patient having a *Staphylococcus aureus* infection, comprising administering to said patient an amount of the agent of claim 32 effective to reduce or inhibit growth or survival of said *Staphylococcus aureus*.
36. A method of protecting a patient against a *Staphylococcus aureus* infection, comprising administering to said patient an amount of the agent of claim 32 effective to prevent said patient from acquiring a *Staphylococcus aureus* infection.
37. The isolated nucleic acid molecule of claim 2, wherein said nucleic acid contains an essential gene.
38. The nucleic acid library of claim 20, wherein said map is in electronic form.
39. The library of claim 39, wherein said electronic form is selected from the group consisting of magnetic storage media, such as a floppy disc, a hard disc storage medium, and a magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; hybrids of these categories such as magnetic/optical storage media; computer readable forms such as a word processing text file, database format, searchable files, executable files and search program software.
40. The transposon insertion map of claim 21, wherein said map is in electronic form.
41. The map of claim 38, wherein said electronic form is selected from the group consisting of magnetic storage media, such as a floppy disc, a hard disc storage medium, and a magnetic tape;

optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; hybrids of these categories such as magnetic/optical storage media; computer readable forms such as a word processing text file, database format, searchable files, executable files and search program software.

42. A method for identifying a library of putative essential or important genes using a High Throughput Transposon Insertion Database (HTTIM), comprising:

- a) mutagenizing a *Staphylococcus* genome with a transposon such that individual cells containing at least one transposon insertion are isolated;
- b) collecting and mapping said at least one transposon insertion in each individual cell so as to form a database of transposon insertion sites, or an HTTIM;
- c) comparing said database of transposon insertion sites with a database comprising the genomic sequence of the bacterium to identify open reading frames in said genomic sequence database that are not disrupted by a transposon insertion; and
- d) forming a library from said putative essential or important genes that are not disrupted by a transposon.

43. The method of claim 42, wherein said bacteria is *S. aureus*.

44. The method of claim 42, wherein said transposon inserts randomly into the target genome.

45. The method of claim 42, wherein said transposon is 3,000 to 6,000.

46. The method of claim 42, wherein said HTTIM comprises at least about 4,000 to 5,000 transposon insertion sites.

47. The library of putative essential or important genes identified by the method of claim 42, wherein said library comprises at most about 500 to 1850 genes.

48. The library of putative essential or important genes identified by the method of claim 42, wherein said library comprises at most about 1000 to 1400 genes.

49. The library of putative essential or important genes identified by the method of claim 42, wherein said library comprises at most about 600-625 genes.
50. The library of putative essential or important genes identified by the method of claim 42, wherein said library comprises at most about 530-543 genes.
51. The method of claim 42, further comprising a statistical calculation for identifying putative essential or important genes.
52. The method of claim 51, further comprising the statistical method applied herein
53. The method of claim 42, further comprising a physical mutagenesis experiment in order to verify essential or important genes.
54. The method of claim 53, wherein said physical mutagenesis comprises knocking out a putative essential or important gene or creating a promoter swap mutant.
55. An essential or important gene identified by the method of claim 53.
56. An antibacterial agent that targets the gene of claim 55, or the gene product encoded by said gene.
57. A pharmaceutical composition comprising said antibacterial agent of claim 56.